

## A Combination of Iontophoresis and the Chelating Agent 1,10 Phenanthroline Act Synergistically As Penetration Enhancers

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**ABSTRACT** The peroxovanadium compound  $\text{VO}(\text{O}_2)_2$  1,10 phenanthroline (bpV(phen)) is capable of lowering blood glucose levels. It is not available in oral form, but it is effective when delivered transdermally. Iontophoresis can significantly reduce the lag time of this response *in vivo* when compared with passive penetration. To better mimic *in vivo* insulin release, we explored the effects of various iontophoretic current durations on dermal penetration of bpV(phen). Iontophoretic transport was not related to total applied charge, as steady-state flux was equivalent for current durations ranging from 15 minutes to 9 hours. We hypothesized that the unexpectedly large transport after just 15 minutes of current was caused by an increase in passive penetration of bpV(phen) induced by iontophoresis. Iontophoretic pretreatment with the chelating agent 1,10 phenanthroline increased passive penetration of bpV(phen), whereas neither the nonchelating isomer 1,7 phenanthroline nor the less potent chelator EDTA were effective. The use of 1,10 phenanthroline as a penetration enhancer for other chemicals was examined with the amino acids alanine and leucine. Fifteen minutes of 1,10 phenanthroline iontophoresis enhances alanine transport 11.4-fold over passive, whereas the 1,7 phenanthroline increased transport by a factor of 4.6 and the iontophoretic control of ethanol by 1.9. Surprisingly, phenanthroline did not enhance  $^3\text{H}$  leucine penetration. The reasons for this selectivity are not clear and warrant further investigation. Overall, the data suggest that chelating agents, specifically 1,10 phenanthroline, may be used as penetration enhancers for the delivery of certain compounds.

**KeyWords:** Transdermal, Skin, Iontophoresis, Vanadium, Diabetes

## INTRODUCTION

A series of peroxovanadium compounds have been synthesized that, when given intravenously, are more potent than other vanadium compounds at lowering blood glucose levels (1). Peroxovanadium compounds contain a central vanadium atom, an oxygen group, 1 or 2 peroxo ligands, and an ancillary bidentate ligand. One of these compounds,  $\text{VO}(\text{O}_2)_2$  1,10 phenanthroline (bpV(phen)), contains the chelating agent 1,10 phenanthroline as its peroxo-ligand (2). This compound is not available in oral form *in vivo*, but it has been delivered transdermally *in vivo* across rat skin in sufficient quantities to lower blood glucose levels by 20% in diabetic animals (in theory the compound would also reduce blood glucose levels in humans) (3).

*In vivo*, insulin, as well as other hormones, is released in a pulsatile fashion. If a continuously high hormonal signal is given, cells become tolerant (desensitized) and lose their ability to respond. We hypothesized that bpV(phen) would be more effective at lowering blood glucose levels when delivered transdermally in a periodic manner. Iontophoresis is an excellent method to control transdermal penetration. The transdermal penetration of bpV(phen) has been performed both passively and iontophoretically (3, 4). A small iontophoretic current, which takes advantage of the molecule's negative charge, is applied to help propel it through the skin, resulting in a faster pharmacodynamic response. The current duration that would provide an optimal dose was therefore examined. However, our results indicate that penetration was not proportional to current duration, prompting an examination of the compound's properties. This article describes the unusual behavior of the peroxovanadium compound, bpV(phen), when different iontophoretic current durations and patterns are applied and attempts to understand how this phenomenon occurs.

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## MATERIALS AND METHODS

### Chemicals

The peroxovanadium compound, bpV(phen), was synthesized as described by Posner et al (2) (Figure 1). All chemicals used for synthesis and buffers were at least reagent grade and were purchased from Sigma Chemical Company (St Louis, MO), Fisher Scientific (St Louis, MO), or Alfa Aesar (Ward Hill, MA). Radiolabeled alanine and leucine were purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

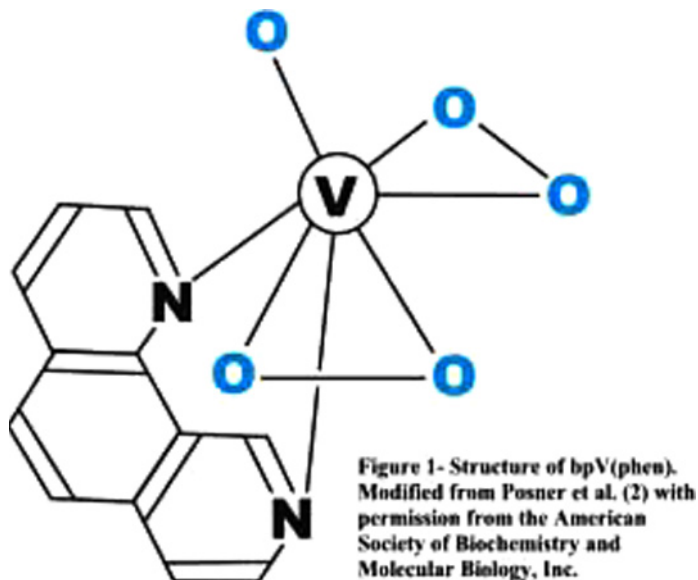


Figure 1. Chemical structure of bpV(phen).

### Experimental Design

Animals were maintained in an Association for the Assessment and Accreditation of Laboratory Animal Care-approved facility at the VA Medical Center, Omaha, NE. Dorsal skin from male hairless mice CRL:SK1, ages 8 to 20 weeks, was removed after the animals were killed by CO<sub>2</sub> asphyxiation. Full thickness skin patches were placed in a Bronough style, flow-through diffusion cell system (PermeGear, Riegelsville, PA). The receptor chamber (0.2 mL) was perfused with 25 mmol Hepes and 133 mmol NaCl at pH 7.4 (1 mL/hr), which then passed to a fraction collector. The skin was allowed to equilibrate for 180 minutes. The receptor chamber (0.2 mL) was perfused at 1mL/hr with a buffer (25 mM Hepes and 133mM NaCl at pH 7.4), which then passed to a fraction collector. The bpV(phen) was dissolved in 25 mM Hepes and 133 mM NaCl at pH 7.4 to a final concentration of 10mM. One mL of the solution was

placed in the donor chamber. The vanadium compound readily binds to Ag/AgCl electrodes, so electrical contact was maintained via salt bridges made from 3% Agarose in 1 M NaCl. The Ag/AgCl electrodes were then connected to a BioRad Model 1000/500 Power Supply (Hercules, CA) in constant current mode set to 0.5 mA/cm<sup>2</sup> (4).

The effect of pulsing was examined by turning the current on for 2 hours and off for 1 hour or on for 1 hour and off for 4 hours. These patterns were repeatedly applied for a total of 9 hours and compared to either 0, 0.25, 4.5, or 9 hours of continuous current.

Another series of experiments was performed to determine if the phenanthroline portion of the bpV(phen) molecule was acting as a penetration enhancer. Either 1,10 phenanthroline, 1,7 phenanthroline, EDTA, or ethanol was placed on the epidermal side of the skin and an iontophoretic current of 0.5 mA/cm<sup>2</sup> was applied for 15 minutes. The pretreatment was then removed and replaced with bpV(phen). Experiments continued overnight with passive penetration. Passive experiments were performed in the presence of 1,10 phenanthroline.

The penetration enhancement properties of 1,10 phenanthroline were examined on 2 radiolabeled amino acids to determine if increases in absorption are specific to bpV(phen) or are a more generalized property of the chelator. An iontophoretic current of 0.5 mA/cm<sup>2</sup> was applied for 15 minutes. The pretreatment was then removed and replaced with either 10 mM alanine (0.33  $\mu$ Ci<sup>14</sup> C-alanine) or 10 mM leucine (0.33  $\mu$ Ci <sup>3</sup>H-leucine) dissolved in 25 mM Hepes and 133 mM NaCl at pH 7.4. Experiments continued with passive penetration, and the receiver fluid was counted in a liquid scintillation counter.

### Assays and Analysis

Vanadium in the form bpV(phen) was assayed using a Beckman 640 spectrophotometer(Fullerton, CA) to quantitate absorbance at 260 nm. Skin placed in an in vitro diffusion chamber, however, will also release molecules that absorb at this wavelength. To account for this, iontophoresis was performed with only buffer in the donor compartment and samples were measured on the spectrophotometer at 260 nm. The data were plotted as control output versus time, and an equation was generated from the results. This curve was

subtracted from the spectrophotometric data obtained when bpV(phen) was present in the donor solution. This technique has been validated by atomic absorption spectroscopy (4). A similar technique was used for the 1,10 and 1,7 phenanthroline experiments.

All data have been expressed as mean + standard error. When comparisons between experiments were conducted, significant differences were assessed by analysis of variance and a Bonferroni posttest at  $P < .05$  using the program Graph Pad Prism (Graph Pad Software, San Diego, CA).

## RESULTS

Iontophoretic delivery of bpV(phen) for different current patterns over 9 hours was determined. The current patterns studied were 1) continuous current; 2) 4.5 hours on and 4.5 hours off; 3) 2 hours on, 1 hour off repeated 3 times; 4) 1 hour on, 4 hours off repeated twice; and 5) no current (passive). Flux patterns for each of the 4 iontophoretic delivery methods were similar even though the total charge applied varied by as much as 3-fold. All 4 patterns resulted in increased penetration when compared with passive delivery (Figure 2).

We hypothesized that the unexpectedly large transport after just 15 minutes of current was caused by an increase in passive penetration of bpV(phen) and that this increase was a result of interactions of phenanthroline with the skin. Therefore, 15 minutes of current were given in the presence of 1,10 phenanthroline, the nonchelating isomer 1,7 phenanthroline, or vehicle (ethanol). The phenanthroline compounds were then removed and passive delivery of bpV(phen) was allowed to proceed (Figure 3). Penetration was significantly greater than for delivery when a control solution was placed on the skin. The 1,7 phenanthroline was not as effective at enhancing penetration as the 1,10 phenanthroline ( $P < .05$ ). These results indicate that the chelating properties cause the penetration enhancement. An additional chelating agent (EDTA) was also used. It did not enhance penetration as effectively as the 1,10 phenanthroline ( $P < .05$ ), suggesting that the relative affinities of 1,10 phenanthroline and EDTA for the various metals that could be involved (eg,  $\text{Ca}^{++}$ ) may explain the difference in properties. An ethanol control, chosen because phenanthroline has limited water solubility and needed to be dissolved in ethanol,

produced results similar to the EDTA, indicating that increased transport attributed to 1,10 phenanthroline is not a result of penetration-enhancing properties of ethanol or to iontophoretic pretreatment.

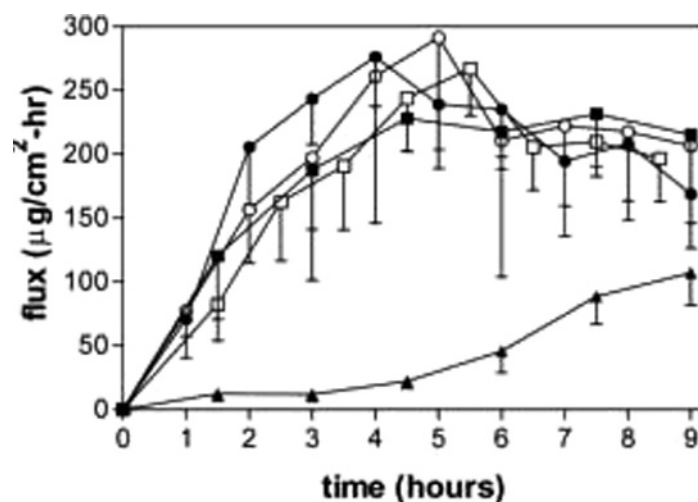


Figure 2. Iontophoretic delivery of the peroxovanadium compound bpV(phen) after the following current patterns: ■ 9 hours on; □ 4.5 hours on, 4.5 hours off; ○ 2 hours on, 1 hour off; ● 1 hour on, 4 hours off; ▲ 0 hour (passive). Each pattern was applied for a total of 9 hours and data are presented as flux versus time. Flux patterns are independent of current duration for the treatments in which current is applied.

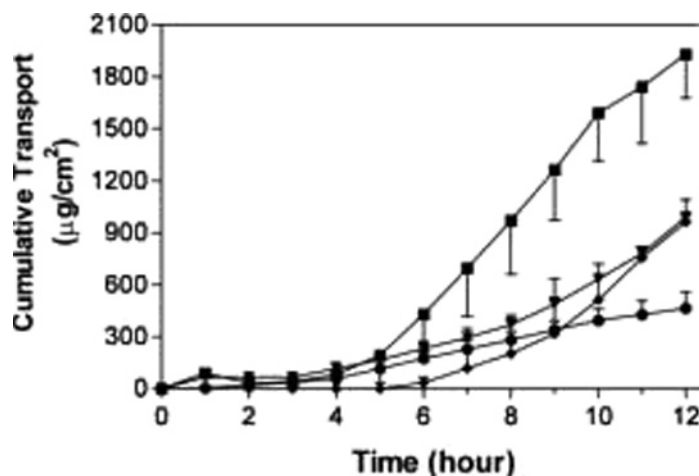
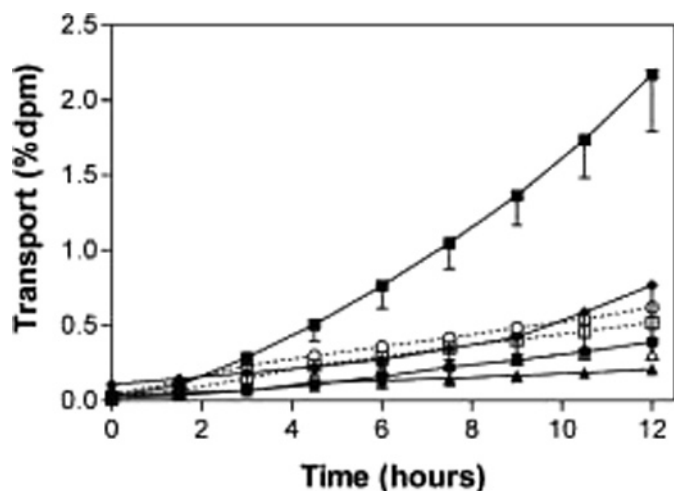


Figure 3. Effect of chelating agents on passive penetration of bpV(phen) after iontophoretic current. Samples were pretreated with the following compounds: ■ 1,10 phenanthroline; ♦ 1,7 phenanthroline; ▲ EDTA; ● ETOH and iontophoresis was applied for 15 minutes. The pretreatments were removed and bpV(phen) was passively applied for 12 hours.

To determine if this was a unique property of the vanadium compound or was more generalized we examined the effect of 1,10 phenanthroline or 1,7 phenanthroline on the transport of 2 amino acids. The data demonstrate that just 15 minutes of iontophoresis of 1,10 phenanthroline enhances alanine transport by 11.4-fold over passive, whereas the 1,7 phenanthroline increased it by a factor of 4.6 and the iontophoretic control of ethanol by 1.9. Surprisingly, the chelating agent did not have the same enhancement effect on the steady-state flux of  $^3\text{H}$  leucine ( $0.58 \pm 0.18 \mu\text{g}/\text{cm}^2$  per hour) when compared with the ethanol control ( $0.68 \pm 0.18 \mu\text{g}/\text{cm}^2$  per hour) (Figure 4).



**Figure 4.** Effect of iontophoretic pretreatment with chelating agents on the transdermal delivery of the amino acids alanine and leucine. ■ 1,10 phenanthroline followed by  $^{14}\text{C}$  alanine; ♦ 1,7 phenanthroline followed by  $^{14}\text{C}$  alanine; ● ETOH followed by  $^{14}\text{C}$  alanine; ▲ passive  $^{14}\text{C}$  alanine only; □ 1,10 phenanthroline followed by  $^3\text{H}$  leucine; ○ ETOH followed by  $^3\text{H}$  leucine; Δ passive  $^3\text{H}$  leucine only.

## DISCUSSION

Iontophoresis has been used to enhance the transdermal delivery of a variety of compounds. Several "rules" generally apply for this process. For example, positively charged molecules tend to have greater transport than their negative counterparts because of the electroosmotic flux that flows from anode to cathode (6). A single positive charge is generally better than more positively charged molecules (7), smaller molecules penetrate better than larger ones, and transport is linearly related to donor concentration (8).

Delgado-Charro et al found an exception to one of these rules when examining iontophoretic transport of

napharelin, an LHRH analog (9). Penetration of this compound actually decreased as the donor concentration increased. Further investigation revealed that napharelin would bind to the skin and its charge would lead to a reversal of the electroosmotic flux. When electroosmotic flow is reversed, penetration decreases. As donor concentration increases, more napharelin is available to bind to the skin, further reducing electroosmotic flux and thus overall penetration.

Another rule of iontophoresis is that drug penetration is linearly related to total charge applied (10). This article reports an exception to this rule. The iontophoretic transport of the peroxovanadium compound bpV(phen) is not a function of the total charge delivered, which, at constant current, is a function of current duration. A brief period of iontophoresis increases passive penetration of the compound, which appears to be related to the chelating agent 1,10 phenanthroline.

These studies began by examining the application of a periodic current, looking for a current pattern that would allow the compound to be delivered in a pulsatile fashion, similar to the release of hormones *in vivo*. This approach was taken by Nakakura et al, who examined the effect of iontophoretic patterns on transdermal delivery of desmopressin acetate. They found that applying a constant amount of charge in varied patterns resulted in different pharmacodynamic responses, and they were able to determine a current pattern that led to the optimal antidiuretic response (11). Previously, we demonstrated that when bpV(phen) was delivered using 4 hours of continuously applied iontophoresis, it was capable of lowering blood glucose levels in diabetic rats (3). Given the results of this study, the current duration necessary to cause a significant decline in glucose level could be reduced substantially.

The current patterns described in this manuscript are very low frequency and are not similar to alternating current (AC) application reported in other works (12). AC current with varying duty cycles has had mixed results when compared with constant current applications (13); some authors reported an improvement in transdermal penetration with periodic current while others did not. The fact that iontophoretically pretreating the skin with ethanol did not enhance penetration to the same level as 1,10

phenanthroline indicates that penetration enhancement induced by the chelating agent is not due to current effects on skin barrier function (14).

The chelation effect is not specific to the vanadium compound because alanine transport was also enhanced. Phenanthroline is also not a universal penetration enhancer because leucine transport was not affected. Obviously, the nature of the transport principle governing the compound delivery will affect the enhancement by phenanthroline. There is a report of o-phenanthroline inhibiting degradation of delta sleep-inducing peptide, leading to increased iontophoretic transport (15). This enhancement was suggested to be a result of inhibition of metalloproteins that degrade the peptide. However, our findings indicate that chelation may also be a factor. The enhancement of alanine, but not leucine, penetration after pretreatment with 1,10 phenanthroline is surprising and we are unsure of the reason for these differences. These results suggest that chelating agents, specifically, phenanthroline may be used as penetration enhancers for the delivery of certain compounds.

## CONCLUSION

The data demonstrate that 1,10 phenanthroline can act as a penetration enhancer without constant current, which may allow a very short current duration to enhance the penetration of some drugs for substantially longer. We hypothesize that the chelating agent 1,10 phenanthroline interacts with divalent metal cations in the skin, making a "pathway" for the remaining peroxovanadium and alanine. The mechanism by which the 1,10 phenanthroline interacts with the skin and the universality of the changes as a penetration enhancer are being examined further.

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